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Evidence for *Gymnosporangium atlanticum* in Europe

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Abstract — Specimens morphologically identical to *Gymnosporangium atlanticum* have been found in Spain at four different locations. We describe these findings and provide an accurate description and illustrations. DNA sequences from these specimens are closely enough related to those from *G. sabinae* that the two species cannot be discriminated with the three DNA markers employed.

Key words — teliospores, *Juniperus*, Morocco, Mediterranean, ITS, TEF1α

Introduction

The genus *Gymnosporangium* R. Hedw. ex DC. (*Pucciniales*) is widespread in temperate regions of the Northern Hemisphere. Its species are obligate parasites of vascular plants, with a complex life cycle that includes several states with different spore types. Most of these taxa are heteroecious, this is, they alternate different biological states in two distinct hosts: the annual aecial state on dicotyledonous host plants (mainly *Rosaceae*), and a perennial telial state on gymnosperms (especially *Juniperus*).

Gymnosporangium atlanticum was first described from specimens in the telial state found on *Juniperus phoenicea* L. growing in dunes at Mehedya, on the Atlantic coast of Morocco (Guyot & Malençon 1957). This species was reported again from Morocco (Rieuf 1969), on *J. phoenicea* and *Juniperus* sp. In his monograph on *Gymnosporangium,* Kern (1973) considered *G. atlanticum* an exclusively African species. To our knowledge, there seem to be no other published records of this taxon except for one citation from China (Zhao &

Zhuang 2006), where specimens on *Juniperus sabina* L. were identified as *G. atlanticum* based on Kern's description.

There is a report by Llimona & Vila (1998) mentioning the occurrence of telia of *G. atlanticum* growing on *Juniperus thurifera* L. and *J. phoenicea* in Retuerta de Pina (Zaragoza, Spain). This report refers to several specimens in the telial state collected by J. Blasco-Zumeta in 1989, 1990, and 1993, which were morphologically identified by one of us (I. Llorens) as *G. atlanticum* and which also match samples found by Llorens in Punta de la Móra (Tarragona, Spain) in 1987, 1989, and 1990 on *Juniperus phoenicea* subsp. *turbinata* (Guss.) Nyman (as "*J. phoenicea* subsp. *lycia*") in sandy soil near sea level. In 2014, 2105, and 2016 new specimens were located in Punta de la Móra on the same host, and in 2016 specimens on *J. phoenicea* were found in two new sites in Tarragona.

The purpose of the present work is to provide morphological and molecular data from these unpublished European collections in order to support future diagnostic and phylogenetic studies in *Gymnosporangium*.

Materials & methods

A piece of the holotype of *G. atlanticum* stored in the herbarium of the Université de Montpelier II, France (MPU), was kindly loaned by Dr. Jean-Michel Bellanger and Dr. Caroline Loup. The Spanish material examined from years 1987 to 1990 is stored in the herbarium CeDoc de Biodiversitat Vegetal, University of Barcelona, Spain (BCN) and the 2016 specimens from La Sénia are in P. Prats personal herbarium (PPR); the remaining specimens are kept at J.L. Fernández personal herbarium (JLF) and are available on request. Microscopic examination was conducted using a Leica

Table 1. Spanish G*ymnosporangium* specimens from *Juniperus* and *Pyrus* hosts used in DNA sequencing, with GenBank accession numbers.

Taxon	Host	Locality	Herb. JLF	LSU	ITS	TEF1α
G. atlanticum	*J. phoenicea* subsp. *turbinata*	Tarragona	20140331-6	–	KM403109	–
			20140331-7	–	KP261044	–
			20140331-10	KM403111	KM403108	–
			20150227-M1	KT160254	KT160250	KT160255
			20150227-M2	KT160253	KT160251	KT160256
G. sabinae	*J. oxycedrus*	Teruel	20140330-3 M2	–	KM403110	KM403112
			20150325-2 M2	–	KT160252	–
G. sabinae	*P. communis*	Teruel	20140923-H2	–	KP261039	–

1000 LED microscope equipped with a EC3 camera; samples from all specimens were mounted in water and additionally in 3% KOH for the holotype and the BCN herbarium material. The teliospores were measured with the Piximètre 5.5 software. Specimens were identified based on the original protologue by Guyot & Malençon and the direct examination of the holotype.

DNA was extracted from eight dry specimens (Table 1), and the polymerase chain reaction (PCR) was performed according to Alvarado et al. (2012). Primers ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993) were employed to amplify the ITS region, while LR0R and LR5 (Vilgalys & Hester 1990) were used for the 28S nLSU region, and EF1-983F and EF1-1567R (Rehner & Buckley 2005) for the translation elongation factor gene (TEF1α). PCR products were checked in 1% agarose gels, and positive reactions were sequenced with the same primers. Chromatograms were checked for putative reading errors, which were corrected.

Taxonomy

Gymnosporangium atlanticum Guyot & Malençon, Trav. Inst. Scient. Chérifien Sér. Bot. 11:15, 1957. Pls 1–3

Telia located on fusiform swellings, in branches of various sizes, erumpent through the bark of the host, also on green stems, often confluent, pulvinate, hemispheric 1.5–3 mm in diameter or elongated ≤2 × 5 mm, 1–2.5 mm high, dark brown, swelling slightly when moist. Teliospores 2-celled, ellipsoidal, apex rounded to conical, base narrowed or sub-rounded, with lipid droplets when young, pedicel cylindrical, hyaline. There are two types of teliospores: the most abundant are slender, constricted at the septum, with fairly homogeneous wall thickness, although often thickened at the apex, light colored, (41–)48.4–48.6(–56) × (16.5–)21.3–21.5(–26) µm, Qm = 2.3; the less abundant teliospores are rounded, slightly or not at all constricted at the septum, provided with a darker and often thinner wall sometimes thickened near the septum, (38–)45.7–46.4(–54) × (22.5–)27.1–27.5(–32) µm, Qm = 1.7; measures including the two types of teliospores are (40.5–)48.1–48.3(–56) × (16–)22–22.1(–28) µm, Qm = 2.2. Wall thickness (from both types of spores): 1.2–3.9 µm. There are intermediate forms between the two main ones already described and also (exceptionally) teliospores with 1 or 3 cells. Germ pores are readily visible; (2–)3–4(–rarely 5) are usually arranged in a more or less narrow band near the septum in both cells, often with 1(–2) additional pores located near the apex or base of the higher or lower cells, respectively. Basidiospores ovoid in front view, broadly ellipsoidal with a straight side or kidney-shaped in side view, apiculate, 15–18 × 9–11 µm, Qm = 1.6.

Ecology & distribution—According to quotes from Morocco, China and Spain, this species develops the telial state in branches of *Juniperus* sect. *Sabina*. In Tarragona, it was found on *J. phoenicea* subsp. *turbinata* in a coastal dune area

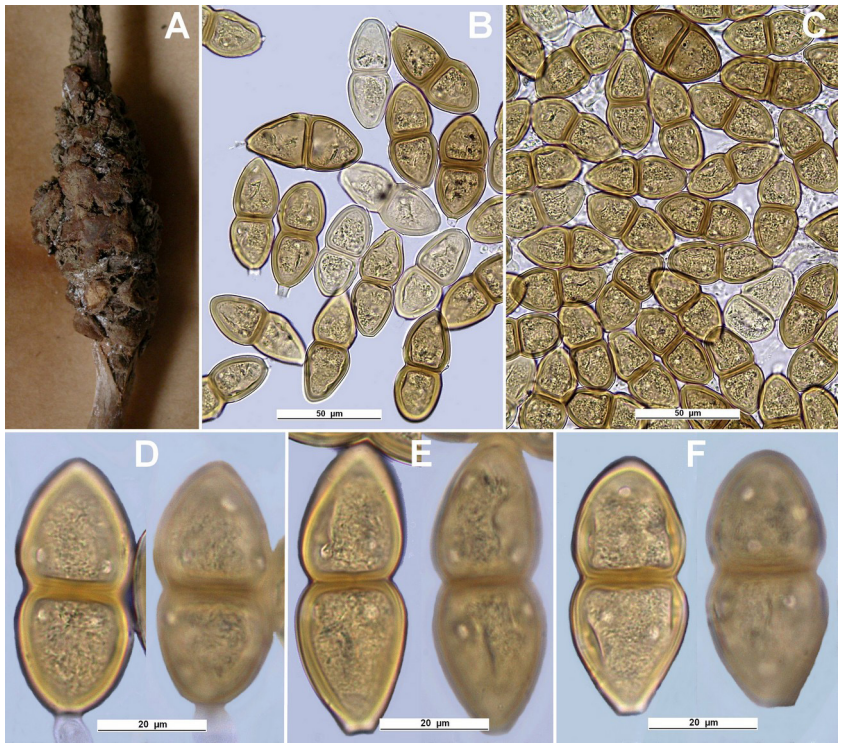

Plate 1. *Gymnosporangium atlanticum,* holotype (MPUC03909). A: dried telia. B, C: teliospores of two types. D–F: arrangement of the germ pores; 4 pores per cell in bands near the septum, with additional pores toward the end of both cells. (A, photo by J-M. Bellanger; B–F, photos by J.L. Fernández).

and on *J. phoenicea* in mountain zones with *Pinus* sp. also present; in Retuerta de Pina, Zaragoza, the hosts were *J. phoenicea* and *J. thurifera* in a Mediterranean steppe habitat. The 24-II-1990 collection from Retuerta de Pina included telia growing on a host galbulus (an unusual substrate for *Gymnosporangium*) but unfortunately the infected galbulus was not conserved in the herbarium specimen. The aecial state of *G. atlanticum* is thus far unknown.

Specimens examined —

Gymnosporangium atlanticum: **MOROCCO**: Mehedya, on *Juniperus phoenicea*, 19-II-1939, leg. Ch. Rungs (holotype MPUC03909). **SPAIN**: **Tarragona**, Punta de la Móra, 0–25 m asl, on *Juniperus phoenicea* subsp. *turbinata* (as "*Juniperus phoenicea* subsp. *lycia*"), 20-II-1987, leg. P. Navarro (BCN-myc ILL 1331); leg. A. Gómez (BCN-myc ILL 1352); leg. M. Tabares (BCN-myc ILL 1353); leg. A. Gómez, P. Navarro et al. (BCN-myc ILL 1377); 20-IV-1989, leg. X. Llimona & N. Pàmies (BCN-myc ILL 1498); 2–4 m asl on *Juniperus phoenicea* subsp. *turbinata,* 31-III-2014, leg. J. Castillo & P. Prats

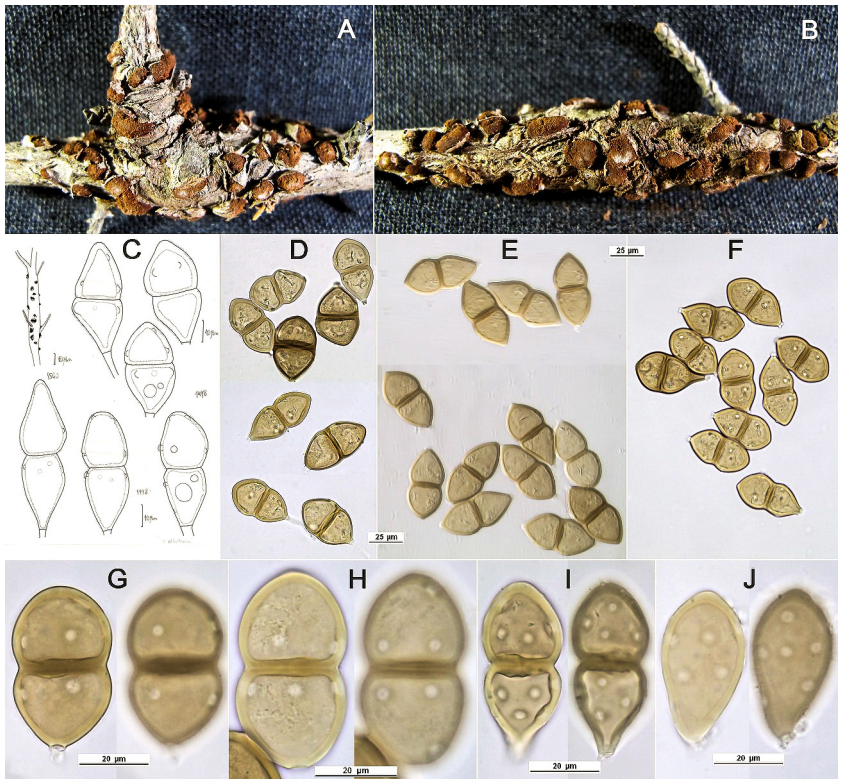

Plate 2. *Gymnosporangium atlanticum,* 1987–1990 collections. A, B: dried telia. C: telia and teliospores (drawing by I. Llorens). D–F: teliospores of two types. G–J: variation in teliospore germ pores (G: 4 pores per cell in bands near the septum; H: bands with 4 and 5 pores, plus 1 per cell towards the apex and base respectively; I: 8 pores in upper cell and 7 in lower cell; J: teliospore 1-celled with 10 pores apparently on 5 parallel planes). (Photos by J.L. Fernández).

(JLF 20140331-3); (JLF 20140331-4); (JLF 20140331-5); (JLF 20140331-6; GenBank KM403109); (JLF 20140331-7; GenBank KP261044); (JLF 20140331-8); (JLF 20140331-9); (JLF 20140331-10; GenBank KM403108, KM403111); (JLF 20140331-11); 04-IV-2014, leg. P. Prats (JLF 20140404-1); (JLF 20140404-2); 27-II-2015, leg. J. Castillo & P. Prats (JLF 20150227-M1; GenBank KT160250, KT160254, KT160255); (JLF 20150227-M2; GenBank KT160251, KT160253, KT160256); (JLF 20150227-M3); (JLF 20150227-M6); (JLF 20150227-M7); (JLF 20150227-M8); (JLF 20150227-M9); (JLF 20150227-M10); 19-III-2015, leg. P. Prats (JLF 20150319-1); Cornudella de Monsant, 850 m asl, on *Juniperus phoenicea*, 27-III-2016, leg. P. Prats (JLF 20160327); La Sénia, 1110 m asl, on *Juniperus phoenicea*, leg. P. Prats (PPR 9584M5). **Zaragoza**, Retuerta de Pina, 390–395 m asl, on *Juniperus thurifera,* 14-V-1989, leg. J. Blasco-Zumeta (BCN-myc ILL 1508); 24-II-1990, leg. J. Blasco-Zumeta (BCN-myc ILL 1557; on *J. phoenicea,* 04-III-1990, leg. J. Blasco-Zumeta; (BCN-myc ILL 1560).

Plate 3. *Gymnosporangium atlanticum,* 2014–2015 collections. A–C: telia (A: moist; B, C: dry). D–F: teliospores of two types (E: germinating teliospore; F: basidium and basidiospore). G–I: germ pores (G: 4 pores per cell in bands near the septum; H, I: with additional pores towards the end of both cells). (A–C, photos by P. Prats; D–I, photos by J.L. Fernández).

***Gymnosporangium sabinae*: SPAIN**: **Teruel**, Beceite, Barranco Comellasos, 595 m asl, on *Juniperus oxycedrus* L., 30-III-2014, leg. J.L. Fernández (JLF 20140330-3 M2; GenBank KM403110, KM403112); 25-III-2015, leg. J.L. Fernández (JLF 20150325-2 M1); (JLF 20150325-2 M2; GenBank KT160252); (JLF 20150325-2 M3); (JLF 20150325-3 M1); (JLF 20150325-3 M2); (JLF 20150325-3 M3); (JLF 20150325-3 M4); (JLF 20150325-3 M5); (JLF 20150325-3 M6); (JLF 20150325-3 M7); Parrizal 30, 579 m asl, on *Pyrus communis* L., 23-IX-2014, leg. J.L. Fernández (JLF 20140923-H2; GenBank KP261039). **Baleares**, Mallorca, Sóller, on *Juniperus phoenicea* subsp. *turbinata*, 18-III-2015, leg. J. Bibiloni (JLF 20150318).

Discussion

Morphological and ecological features of the Spanish collections match those of the protologue of *Gymnosporangium atlanticum* as well as our own observations of the holotype. We believe that all these specimens represent a single taxon, which we regard as the first report of *G. atlanticum* in Spain and Europe.

Three other *Gymnosporangium* species produce teliospores with more than two pores per cell: *G. multiporum* F. Kern from USA teliospores have 5–7 scattered pores, but no special pattern can be observed (Kern 1909), while *G. gjaerumii* Korbonsk. & Azbukina from Asia teliospores have 4 pores located more or less close to the septum (Azbukina 1997). Both *G. multiporum* and *G. gjaerumii* develop telia on juniper leaves while the telia of *G. atlanticum* occur in branches. Finally, *G. taianum* F. Kern from Asia resembles *G. atlanticum* in the number and distribution of the teliospore pores, but its host is *Cupressus* (Kern 1964).

We hope that our DNA sequences, the first molecular data from morphologically validated specimens of *G. atlanticum,* will help identify its aecial state in the future. A BLAST search of the ITS region from *G. atlanticum* revealed a 99% identity with several sequences identified as *G. sabinae* (Dicks.) G. Winter from an unpublished work by Filipp & Spornberger (seq. KF925316–KF925321) but only 86% identity with a sequence (KJ720183) from

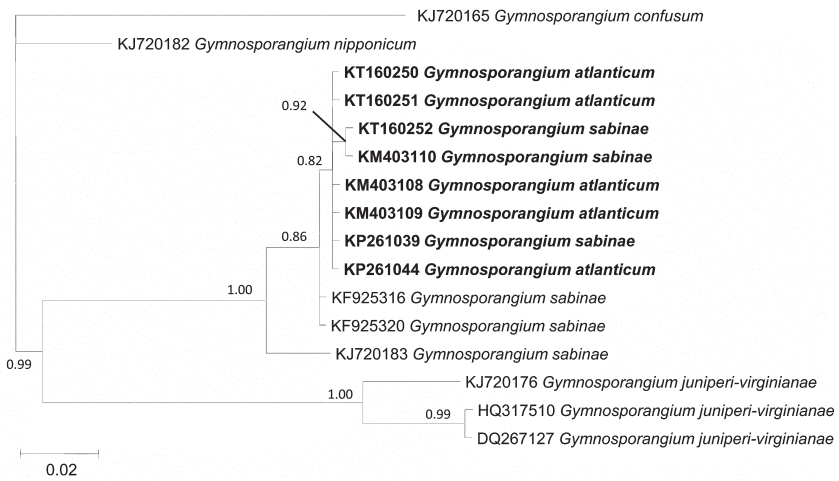

Plate 4. Consensus phylogram constructed in MrBayes 3.1 from an alignment of the ITS sequences produced with those most closely related in public databases. Nodes with >0.80 PP support are annotated. Sequences highlighted in bold have been produced in the present work.

Plate 5. *Gymnosporangium sabinae*. Top: moist telia. Bottom: teliospores of two types, and basidiospores (mostly out of focus). (Photos by J.L. Fernández).

another specimen of *G. sabinae* from Novick et al. (unpubl.) (Plate 4). LSU data revealed subtle differences with a sequence from Novick et al. (unpubl.), and other *G. sabinae* sequences from a Turkish specimen found on *Pyrus* sp. (Dervis et al. 2010). TEF1α sequences found no close BLAST match, but only a 87% similarity with a *G. clavariiforme* sequence (DQ925242) from van der Merwe et al. (2007). Attempts to obtain DNA sequences from the *G. atlanticum* holotype and the specimens collected from 1987 to 1990 failed.

Our comparison of sequences from specimens matching our own concept of *G. sabinae* with those of *G. atlanticum* revealed almost no ITS differences, with only a few positions exhibiting heteromorphisms or ambiguous base readings. LSU was entirely identical between our samples of *G. sabinae* and *G. atlanticum*, while TEF1α presented only scattered heteromorphic sites or

ambiguous reads. Unfortunately, the RPB2 gene could not be sequenced with primers bRPB2-6F2 and fRPB2-7cR (Matheny et al. 2007).

These sequence analyses suggest that *G. atlanticum* and *G. sabinae* are extremely closely related, possibly indicating a recent speciation event. To test this hypothesis, more DNA markers with higher phylogenetic signal should be studied. Spanish specimens that might represent a different species from those occurring in Morocco should be examined first before drawing any taxonomic conclusion.

Gymnosporangium atlanticum and *G. sabinae* share similar ecologies: *Juniperus phoenicea*, *J. phoenicea* subsp. *turbinata, J. thurifera,* and *J. sabina* are hosts of the telial states in both. Morphologically, however, *G. sabinae* (Plate 5) and *G. atlanticum* are very different. The telia differ largely in shape and size: in *G. sabinae* they are conic laterally compressed or tongue-shaped and ≤10(–15) mm high when moist. Most *Gymnosporangium* species (possibly all) produce two distinct kinds of teliospores that differ more or less in shape and in wall color (Kern 1973). In *G. sabinae*, unlike *G. atlanticum*, the slender and light colored teliospores usually have thinner walls than the rounded and dark colored teliospores. The teliospores of *G. sabinae* are only slightly constricted at the septum and have only two germ pores per cell near the septum, while the teliospores of *G. atlanticum* are clearly constricted at septum and have up to 6 germ pores per cell with different arrangement.

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